AWARD NUMBER: **W81XWH-15-1-0300**

TITLE: **Effects of Radiation on the Microbiota and Intestinal Inflammatory Disease**

PRINCIPAL INVESTIGATOR: **Dr. David Underhill**

CONTRACTING ORGANIZATION: **Cedars-Sinai Medical Center**
Los Angeles, CA 900478

REPORT DATE: **Sept 2016**

TYPE OF REPORT: **Annual**

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2016	Annual	31 Aug 2015 - 30 Aug 2016

4. TITLE AND SUBTITLE

Effects of Radiation on the Microbiota and Intestinal Inflammatory Disease

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-15-1-0300

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Stephen Shiao, MD/PhD (Initiating PI)
David Underhill, PhD (Collaborating PI)

E-Mail: Stephen.Shiao@cshs.org; David.Underhill@csmc.edu

5d. PROJECT NUMBER

5e. TASK NUMBER
–

5f. WORK UNIT NUMBER
–

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Cedars-Sinai Medical Center
8700 Beverly Blvd.
Los Angeles, CA 90048

8. PERFORMING ORGANIZATION REPORT NUMBER
–

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)
–

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
–

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
–

14. ABSTRACT

In this annual report (covering initiating and collaborating PI projects) we report the completion of initial experiments investigating the effect of whole body and focal (GI tract) irradiation of mice on the bacterial and fungal microbiota. We have identified substantial changes in intestinal microbial communities induced by both types of radiation exposure. In particular, specific fungal community changes appear to be pronounced and relatively long-lived. As outlined in the project proposal, we are now embarking on experiments aimed a evaluating the effects of these changes on intestinal susceptibility to inflammatory disease.

15. SUBJECT TERMS

Radiation, microbiome, mycobiome, colitis, cancer

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	16	19b. TELEPHONE NUMBER *(include area code)* –

Table of Contents **Page**

1. INTRODUCTION

Exposure of the intestines to radiation may occur through unintended exposure from events such as nuclear accidents or through deliberate exposure to radiation such as during treatment for cancer. While a serious nuclear event might lead to many fatalities, an even larger number of people would be exposed to sublethal doses of radiation. These people, as well as patients who receive pelvic or abdominal radiation as part of their cancer treatment, often manifest bowel symptoms of diarrhea, and many people, even those with minimal acute symptoms, will develop long-term consequences of irradiation including permanent changes to bowel function and intestinal fibrosis, which can cause strictures or even bowel obstructions. It has been estimated that as many as 90% of patients receiving pelvic radiation experience long-term effects on gastrointestinal health, with over 50% reporting that the changes significantly degrade quality of life. The etiology of radiation-induced bowel toxicity has been linked to changes in the microvascular structure of the gastrointestinal tract, but increasing evidence suggests a role for immune cells associated with the intestine and their interactions with the normal microbial contents of the gut.

2. KEYWORDS

Radiation, microbiome, mycobiome, colitis, cancer.

3. ACCOMPLISHMENTS

What were the major goals of the project?

Below is the Statement of Work (SOW) covering the period of the annual review. Completion milestones are indicated.

Specific Aim 1: Define the alterations in gut microbiota (bacterial & fungal) in mice exposed to total body irradiation (TBI) or focal radiation to the GI tract.	**Timeline**	**Status**	**Site 1** (Stephen Shiao, MD, PhD)	**Site 2** (David Underhill, PhD)
Major Task 1: Effects of whole body radiation on bacterial and fungal microbiota.	Months			
Subtask 1: Expose mice (10/group) to <u>whole body</u>, <u>low dose</u> radiation & monitor weight loss & collect fecal pellets over 60 days. (40 animals)				
• Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens.	4-5	Completed (Jan. 2016)	Dr. Shiao	
• Evaluate bacterial/fungal diversity in all fecal samples.	5-6	Completed (Jun. 2016)		Dr. Underhill
Subtask 2: Expose mice (10/group) to <u>whole body</u>, <u>high dose</u> radiation & monitor weight loss & collect fecal pellets over 60 days. (40 animals)				
• Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens.	5-6	Completed (Mar. 2016)	Dr. Shiao	
• Evaluate bacterial/fungal diversity in all fecal samples.	7-8	Completed (Aug. 2016)		Dr. Underhill
Subtask 3: Lock in fungal database & Train new staff.	1-3	Completed (Oct. 2015)		Dr. Underhill

Subtask 4: Expand repertoire of microbe-specific PCR primers to be used in the subsequent analyses. Train new staff.	1-3	Completed (Oct. 2015)	Dr. Shiao	
Local IRB/IACUC Approval	0	Completed (Aug. 2015)		
Milestone #1A: ACURO Approval.	4	Completed (Dec. 2015)	Dr. Shiao	
Milestone #1B: Database fixed and made available on website.	6	Completed (Nov. 2015)		Dr. Underhill
Major Task 2: Effects of focal radiation on bacterial and fungal microbiota.				
Subtask 1: Expose mice (10/group) to <u>abdominal</u>, <u>low dose</u> RT & monitor weight loss & collect fecal over 60 days. (40 animals) • Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens. • Evaluate bacterial/fungal diversity in all fecal samples.	6-7 7-8	Completed (May 2015) Completed (June 2015)	Dr. Shiao	Dr. Underhill
Subtask 2: Expose mice (10/group) to <u>abdominal</u>, <u>high dose</u> RT & monitor weight loss & collect fecal over 60 days. (40 animals) • Perform radiation exposure, collect endpoint tissue for histological examination, evaluation of immune cell infiltration, PCR of microbial burdens. • Evaluate bacterial/fungal diversity in all fecal samples.	7-8 8-9	Completed (July 2015) Completed (Aug. 2015)	Dr. Shiao	Dr. Underhill
Milestone #2A: Complete processing & analysis of first 160 animals (effects of different types of radiation on the microbiome). Expect to find significant changes in bacterial, fungal, & immune parameters. *Milestone #2B: Co-author manuscript on the effects of radiation on the intestinal microbiota.*	12 10-16	Ongoing Ongoing	Dr. Shiao	Dr. Underhill
Specific Aim 2: Investigation of radiation-induced changes in sensitivity to a representative selection of murine models of intestinal inflammatory challenge.				
Major Task 1: Investigation of radiation-induced changes in sensitivity to DSS colitis				
Subtask 1: Expose mice (10/group) to <u>abdominal</u>, <u>low dose</u> RT & induce colitis with DSS. Monitor weight loss and collect fecal pellets for 12 days following exposure. (80 animals) • Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens. • Evaluate bacterial/fungal diversity in all fecal samples.	9-10 10-11	In progress In progress	Dr. Shiao	Dr. Underhill
Subtask 2: Expose mice (10/group) to <u>abdominal</u>, <u>high dose</u> RT & induce colitis with DSS. Monitor				

weight loss and collect fecal pellets for 12 days following exposure. (80 animals) • Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens. • Evaluate bacterial/fungal diversity in all fecal samples.	11-12 12-13	In progress In progress	Dr. Shiao	 Dr. Underhill
Milestone #3A: Complete analysis of initial radiation-induced changes in DSS model. Expect to find significant changes in bacterial, fungal, & immune parameters.	16	Not yet started	Dr. Shiao	Dr. Underhill
	Major Task 2: Investigation of radiation-induced changes in sensitivity to TNBS colitis & T cell transfer colitis			
Subtask 1: Expose mice (10/group) to abdominal, low dose RT & induce colitis with TNBS or $CD4^{+}CD45RB^{high}$ T cells. Monitor weight loss and collect fecal pellets over 12 days. (80 animals) • Perform radiation exposure, collect endpoint tissue for histology, inflammation measurements & PCR of microbial burdens. • Evaluate bacterial/fungal diversity in all fecal samples.	13-14 14-15	Not yet started Not yet started	Dr. Shiao	 Dr. Underhill

What was accomplished under these goals?

1) Major Activities

During this period from September 2015 – August 2016, **we completed both Major Task 1 and 2 for Specific Aim 1** as outlined in the statement of work (SOW). More specifically, we accomplished the following:

- We revised and completed the fungal database for analysis of our fecal samples (Subtask 3 and Subtask 4, Milestone 1B). This database included several new species identified during our pilot studies as well as expanding on some of the existing identifiers within the database.

- Attained ACURO approval of our mouse protocol (Milestone 1A)

- We completed experiments comparing the effects of both high and low dose whole body radiation on bacterial and fungal microbiota (Major Task 1, Subtasks 1 and 2)

- We also completed experiments comparing the effects of both high and low dose focal abdominal radiation on bacterial and fungal microbiota (Major Task 2, Subtasks 1 and 2)

2) Specific Objectives

Following ACURO approval of our mouse protocol in December 2015, we initiated our mouse experiments. In a series of 4 large experiments (Major Task 1, Subtasks 1 and 2), we compared two different doses of total body irradiation (TBI). We collected fecal samples throughout the course of the experiment to analyze the changes in the microbiome following TBI. At the end of the experiment, we also harvested the intestines and mesenteric lymph nodes for multiparametric flow cytometry and histology to assess changes in the intestinal immune composition. We then completed an additional 4 experiments in which we compared two different doses of abdominal only radiation. Again, we collected fecal samples throughout the experiments and intestinal samples

at the end of the experiment for assessment of changes in the microbiome and intestinal immune composition respectively (Major Task 2, Subtasks 1 and 2).

We then generated DNA from fecal samples collected throughout the experiment and analyzed then for overall bacterial and fungal content using quantitative PCR and sequenced the fecal samples to identify specific species. We are currently in the process analyzing the sequencing data.

3) Significant Results/Key Outcomes

From our first set of experiments, we observed that following high dose (8 Gy) TBI that mice lose significantly more weight compared to low dose (2 Gy) TBI and that the high dose group had a prolonged delay in weight recovery following RT (**Figure 1A**). This weight loss pattern was also mirrored in the low and high dose abdominal RT only groups though both the abdominal only groups experienced less overall weight loss compared to the TBI groups (**Figure 1B**).

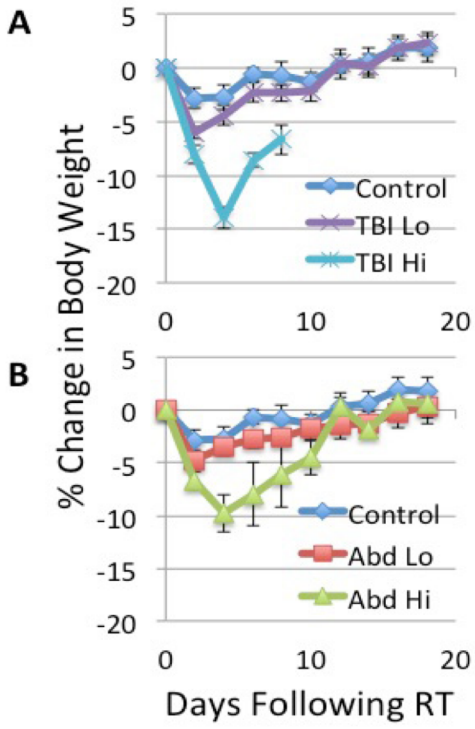


Figure 1. RT induces weight loss following either TBI or abdominal RT.

We found that over the course of the experiments that the bacterial content for both the TBI groups and the high dose abdominal group drops sharply compared to controls and took several weeks to recover to pre-treatment levels (**Figure 2**). Sequencing data demonstrated that both TBI and abdominal RT produced marked changes in the populations of bacteria and fungi in the stool. Interestingly, RT appears to change the landscape such that different species become dominant rather than a global decrease in increase in all populations (**Figure 3**).

Accompanying these changes in the micro- and mycobiome, we also found that there were significant changes in the $CD4^+$ and $CD8^+$ T cells, regulatory T cells, macrophages and dendritic cells in both the colon lamina and mesenteric LN with TBI (**Figure 4**). High-dose TBI appears to reduce $CD4^+$ and $CD8^+$ T cells almost completely while having a more modest depleting effect on regulatory T cells. Further, there are more $CD11b^+$ monocytes, but decreased $F4/80^+$ macrophages in both the colon lamina and mesenteric LN. Interestingly, you see an increase in the number of $CD11c^+$ DC in the mesenteric LN with high dose abdominal RT and low dose TBI, but only modest changes with high-dose TBI.

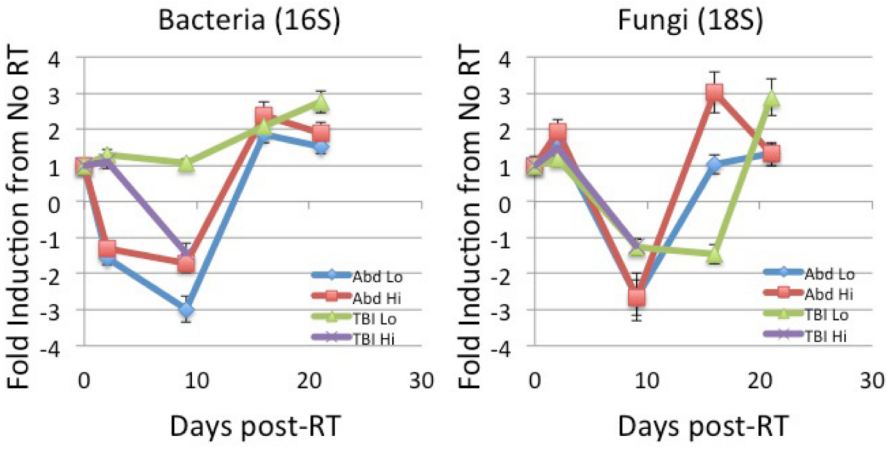


Figure 2. Both TBI and abdominal RT lead to loss of bacteria and fungi.

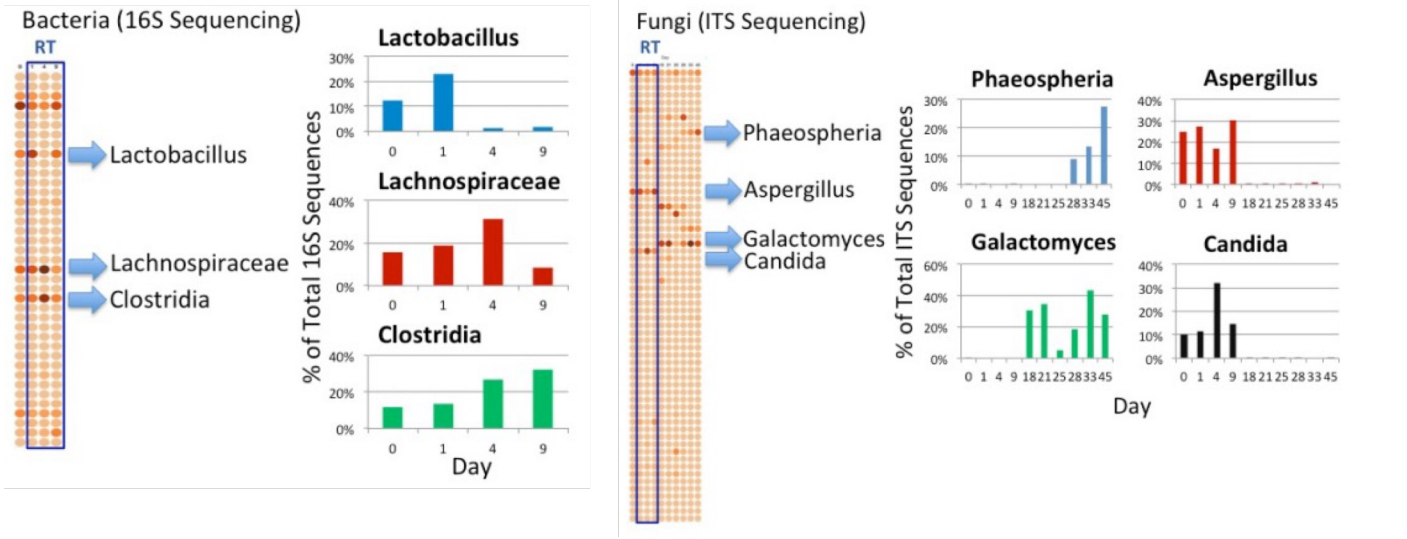


Figure 3. 16S and ITS sequencing show changes in both bacterial and fungal species following high-dose TBI.

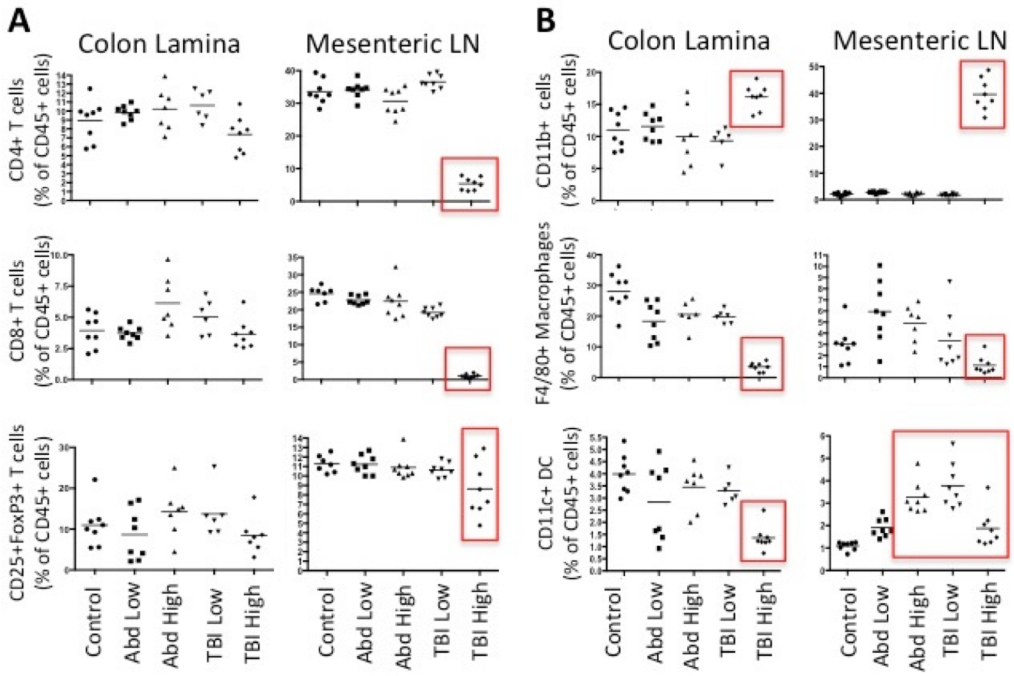


Figure 4. High-Dose TBI induces significant inflammatory changes in the colon lamina and mesenteric lymph nodes that is not seen with either low dose TBI or focal abdominal RT.

Though the analysis is currently ongoing, from our current data, we conclude that TBI and abdominal RT have significantly different effects on the bacterial and fungal populations in the intestine. Further, we also find that there are significant effects of high-dose TBI on the immune composition of the colonic lamina and mesenteric LN that are not seen with either low-dose TBI or focal abdominal RT.

4) other achievements.

In addition to our experimental accomplishments, we also had the opportunity to update the fungal database to include several new species of fungi we identified and post this database online for our other projects and for other groups to access.

What opportunities for training and professional development has the project provided?

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?

The project will continue as planned following the discussion in the text of the proposal and the experimental plans outlined in the Statement of Work. No substantial changes to this plan are currently anticipated.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

It is well-known that abdominal exposure to radiation often has intestinal consequences including diarrhea and intestinal inflammation and can lead to long-term disruption of normal bowel function and fibrosis. Less clear to date is the effect of radiation on the intestinal microbiome. A growing theme in our understanding of intestinal inflammation is that it is strongly dependent on the makeup of the microbiome and interactions of the host immune system with these organisms. Some prior human and animal studies had suggested that whole body radiation could affect intestinal bacterial populations. However, nothing has been known about how radiation exposure affects fungal communities in the gut, and nothing has been known about how radiation-induced changes in the microbiota may be associated with susceptibility to animal models of intestinal inflammatory disease.

As described in the outline of accomplishments above, in the first year of this project we have already made substantial new discoveries. Radiation exposure in mice results in profound changes in the fungal microbial population (as well as causing more modest changes in bacterial populations), and intestinal inflammation is exacerbated in the DSS model of colitis.

What was the impact on other disciplines?

This project has supported the development and refinement of a unique manually curated fungal database. Characterization of microbiomes by high-throughput sequencing of microbial rDNA requires comparison of sequences recovered from a sample to a database linking those sequences to specific species of organisms. For bacteria, a long-standing effort has produced a well-accepted and commonly-used database of sequences. For fungi, this is more complicated and a “standard” database has not been available. We have generated a database used in this study that performs well at identifying fungal sequences in intestinal samples. It is expected that this database will be used widely by other groups in studies of intestinal microbiota as well as in studies of microbiomes at other sites.

What was the impact on technology transfer?

Nothing to Report.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to Report.

Changes that had a significant impact on expenditures

The rate of expenditures was a little low in the first half of this first year as we hired and trained staff and sought regulatory approvals. The current rate of expenditures is accelerated and projected to make up the differences over years 2 and 3.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to Report.

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals.

Nothing to Report.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS

Publications, conference papers, and presentations

Nothing to Report.

Website(s) or other Internet site(s).

https://risccweb.csmc.edu/microbiome/thf/
This is the publically-available download site for the fungal ITS "Targeted Host Fungi" (THF) database.

Technologies or techniques.

Nothing to Report.

Inventions, patent applications, and/or licenses.

Nothing to Report.

Other Products.

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

1) PDs/PIs.

Name:	*Stephen Shiao, M.D./Ph.D.*
Project Role:	*Initiating PI*
Researcher Identifier (e.g. ORCID ID):	*orcid.org/0000-0001-7586-2885*
Nearest person month worked:	*Project #1: 2.5*
Contribution to Project:	*Dr. Shiao is the PI of project #1. He is responsible for overseeing all of the animal studies including radiation exposure, tissue harvesting, and immunophenotyping. He is responsible for managing all of the personnel participating in project #1.*

Funding Support:	*Funding for these activities were provided by this award.*

Name:	*David Underhill, Ph.D.*
Project Role:	*Collaborating PI*
Researcher Identifier (e.g. ORCID ID):	*orcid.org/0000-0002-2989-658X*
Nearest person month worked:	*Project #2: 2*
Contribution to Project:	*Dr. Underhill is the PI of project #2. He is responsible for microbiome characterization in mouse tissue samples using high-throughput DNA sequencing of ribosomal genes. He is responsible for curating the fungal ITS database and for managing all of the personnel participating in project #2.*
Funding Support:	*Funding for these activities were provided by this award.*

2) Other personnel.

Name:	*Jose Limon, PH.D.*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*Project #1: 6* *Project #2: 6*
Contribution to Project:	*Dr. Limon is a postdoctoral fellow working (50%) with Dr. Shiao on project #1 and (50%) with Dr. Underhill on project #2. He is performs the animal models of colitis and harvests tissue for analysis (project #1). He prepares DNAs and performs quality assurance test in preparation for sequencing of ribosomal DNAs (project #2).*
Funding Support:	*Funding for these activities were provided by this award.*

Name:	*Paul Noe, B.S.*
Project Role:	*Research Associate*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*Project #1: 6*
Contribution to Project:	*Mr. Noe is a laboratory technician who has been involved in performing animal experiments in Project #1.*
Funding Support:	*Funding for these activities were provided by this award.*

Name:	*Viviana Maymi, B.S.*
Project Role:	*Research Associate*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*Project #1: 3*
Contribution to Project:	*Ms. Maymi is a laboratory technician who has been involved in performing animal experiments in Project #1.*

Funding Support:	*Funding for these activities were provided by this award.*

Name:	*Xiaoshan Shirley Shi*
Project Role:	*Postdoctoral Fellow*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*Project #1: 4*
Contribution to Project:	*Dr. Shi is a is a postdoctoral fellow working (100%) with Dr. Shiao on project #1 who started in June 2016 (hence the 4 months). In conjunction with Dr. Limon-Tello she performs the animal models of colitis and harvests tissue for analysis (project #1).*
Funding Support:	*Funding for these activities were provided by this award.*

Name:	Jie Tang, Ph.D.
Project Role:	*Genomics & Bioinformatics support*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*Project #2: 1*
Contribution to Project:	Dr. Tang is the acting director of the Cedars-Sinai Genomics core facility (replacing Dr. Vincent Funari), and has been instrumental in coordinating sequencing-based microbiome analyses in Project #2.
Funding Support:	*Funding for these activities were provided by this award.*

Name:	Vineela Gangalapudi, Ph.D.
Project Role:	*Bioinformatician*
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*Project #2: 5*
Contribution to Project:	*Dr. Gangalapudi is a talented bioinformatician who has joined the Cedars-Sinai Genomics core to take the place of Dr Tang when he became director. She has been responsible for processing the high volume of sequencing data generated by project #2.*
Funding Support:	*Funding for these activities were provided by this award.*

Name:	Matthew Gargus
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*Project #2: 5*
Contribution to Project:	*Mr. Gargus is a laboratory technician who is responsible for processing samples for analysis in Project #2.*

Funding Support:	*Funding for these activities were provided by this award.*

Name:	Christian Leal
Project Role:	Research Associate
Researcher Identifier (e.g. ORCID ID):	*N/A*
Nearest person month worked:	*Project #2: 1*
Contribution to Project:	*Mr. Leal was a laboratory technician who is contributed to processing samples for analysis in Project #2.*
Funding Support:	*Funding for these activities were provided by this award.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Stephen Shiao, MD/PhD

Retired Support

Mann-Whitney-Eiger Award (Shiao) CTSI Scholar Seed Grant 09/01/14 – 09/01/15
"Influence of the Microbiome on the Efficacy of RT"
To examine the effect of the bacterial and fungal microbiome on the post-radiation anti-tumor immune response in a murine model of breast cancer.
Role: PI, 1% FTE, Total Direct+Indirect 1yr award: $30K
Grant Officer: Denis Magoffin (denis.magoffin@cshs.org)
No Overlap

Ongoing Support (No change or reduced effort)

Junior Faculty Award (Shiao) American Society of Radiation Oncology 7/1/14 – 6/30/2016 (currently no-cost extension (NCE))
"The Impact of Macrophage Polarization on the Efficacy of Radiation Therapy"
To define the effect of targeting macrophage bioeffector function in the anti-tumor immune response in a murine model of breast cancer.
Role: PI, 2.63% FTE, Total Direct+Indirect 2yr award: $200K
Grant Officer: Crystal Carter (research@astro.org)
No Overlap

K08 CA1191139 (Shiao) NIH/NCI 07/15/15 - 06/30/20
"The Impact of Macrophage Polarization on the Efficacy of Radiation Therapy"
To investigate the mechanisms of enhanced efficacy of radiation therapy with IL-4 blockade in a murine model of breast cancer.
Role: PI, 75% FTE, Total Direct+Indirect Requested 5yr award: $883K
Grant Officer: Susan Perkins (susan.ciolino@nih.gov)
No Overlap

David Underhill, Ph.D.

Retired Support

R21 AI103471 (Underhill) NIH/NIAID 2/1/2014 – 1/31/2016

"Measuring Phagosomal Temperatures"
To investigate the role of temperature in regulating formation and maturation of phagosomes in macrophages and dendritic cells.
Role: PI, 5% FTE, Total Direct+Indirect 2yr award: $422K
Grant Officer: Helen Quill (Hquill@niaid.nih.gov)
No Overlap

Senior Investigator Award (Underhill) Crohn's and Colitis Foundation 7/1/12 – 6/30/2015
"Anti-Fungal Immunity in Ulcerative Colitis"
To define the mycobiome in patients with ulcerative colitis and to explore associations with disease severity and functions of Dectin-1 polymorphisms.
Role: PI, 8% FTE, Total Direct+Indirect 3yr award: $347K
No Overlap

Ongoing Support (no change or reduced effort)

R01AI071116 (Underhill) NIH/NIAID 7/1/06 – 6/30/2018
"Dectin-1 Signaling Mechanisms"
To define the molecular and cellular mechanisms of signaling by the anti-fungal innate immune receptor Dectin-1.
Role: PI, 15% FTE, Total Direct+Indirect 4yr award: $1.8M
Grant Officer: Thomas Palker (palkert@niaid.nih.gov)
No Overlap.

R01 GM085796 (Underhill) NIH/NIGMS 4/1/12 – 3/31/2016 (currently no-cost extension (NCE))
"Innate Immune Sensing of Bacterial Sugars"
To define the innate immune mechanisms by which macrophages and dendritic cells detect bacterial cell walls.
Role: PI, 20% FTE (currently NCE reduced to 1%), Total Direct+Indirect 4yr award: $1.29M
Grant Officer: Sarah Dunsmore (dunsmores@nigms.gov)
No Overlap

R01 DK093426 (Underhill) NIH/NIDDK 7/1/12 – 6/30/2016 (currently no-cost extension (NCE))
"Host immunity to commensal gut fungi"
To define the roles of pathogenic fungi and the anti-fungal immunity genes for Dectin-1 and CARD9 in intestinal inflammation. There is no study of radiation in this project.
Role: PI, 15% FTE (currently NCE reduced to 1%), Total Direct+Indirect 4yr award: $1.78M
Grant Officer: Peter Perrin (Peter.Perrin@nih.hhs.gov)
No Overlap

New Support
PO1 DK046763 (Targan) NIH/NIDDK 9/2/16 – 8/31/2021
"IBD: Role of Genetic and Immunopathologic Mechanisms"
"Project 4: Immune Responses to Fungi Associated with Crohn's Disease (Project PI: Underhill)"
The project aims to understand the mechanisms of interaction of Crohn's disease-associated fungi *Malassezia* and *Aureobasidium* with the gut immune system.
Role: PI, 10% FTE, Total Direct+Indirect 5yr project 4 award: $2.1M
Grant Officer: Robert Karp (karpr@extra.niddk.nih.gov)
No Overlap.

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

This is a collaborative award. Independent, but identical annual reports are filed. Contributions of each of the two projects and personnel have been indicated throughout the report.

QUAD CHARTS:

An updated quad chart has been included.

9. APPENDICES

1. Updated Quad Chart

Effects of radiation on the microbiota and intestinal inflammatory disease

Proposal No. PR140839/PR140839P1

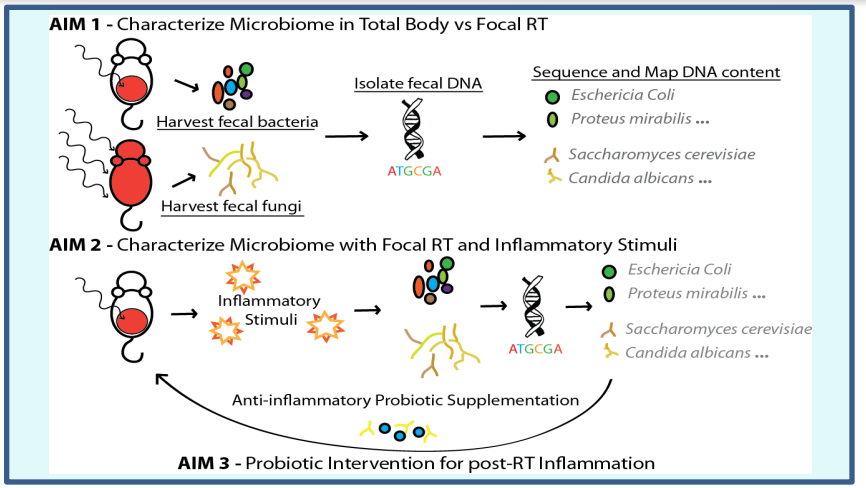
PI: Stephen Shiao MD PhD, David Underhill, PhD **Org:** Cedars-Sinai Medical Center **Award Amount:** $1,500,000.00

Study/Product Aim(s)

- **Aim 1:** Characterize the alterations in gut microbiota (bacterial & fungal) in mice exposed to total body irradiation (TBI) or focal radiation to the GI tract.
- **Aim 2:** Investigation of radiation-induced changes in sensitivity to a representative selection of murine models of intestinal inflammatory challenge.
- **Aim 3:** Manipulation of the intestinal microbiota to affect inflammation exacerbated by radiation exposure.

Approach

We will use immunohistochemistry, flow cytometry and next generation sequencing techniques in a murine model of gut irradiation to test the hypothesis that specific alterations in the microbial composition within the gut leads to increased sensitivity to inflammatory stimuli following intestinal exposure to radiation.



Accomplishment: We completed the fungal database allowing for identification of the fecal fungal species. Furthermore, we have also found differences in the bacterial and fungal populations between total body irradiation and focal abdominal radiation.

Timeline and Cost

Activities CY	15	16	17	18
Characterize changes in microbiome and gut immune cell composition in total body vs focal RT				
Delineate changes in microbiome and gut immune cell composition following RT and various inflammatory stimuli				
Investigate effect of altering the microbiome on the development of post-RT intestinal sensitivity				
Estimated Budget ($1.5 mi)	**$200K**	**$500K**	**$500K**	**$300K**

Updated: Sept 15, 2016

Goals/Milestones

CY15 Goal – Effects of total body irradiation vs focal RT on intestine
☑ACURO Approval, staff hired/trained (**COMPLETED**)
☑Fungal/Bacterial database available (**COMPLETED**)
CY16 Goals – Effects of total body irradiation (TBI) vs focal RT on intestine
☑Characterization TBI vs focal RT on bact/fung microbiota (**COMPLETED**)
❑Analysis of microbiome changes in irradiated guts and DSS
CY17 Goal – RT-induced changes in gut sensitivity
❑Analysis of microbiome changes in irradiated guts in other colitis models and infectious organisms
❑Analysis of effects of bacterial/fungal depletion on gut sensitivity to RT
CY18 Goal – Intervention studies to alter RT-induced gut sensitivity
❑Analysis of effects of lactobacillus and saccharomyces supplementation on on gut sensitivity to radiation

Comments/Challenges/Issues/Concerns

- None

Budget Expenditure to Date (Shiao/Underhill)

Projected Expenditure (direct + F&A): $424,995/$424,259
Actual Expenditure (direct + F&A): $309,155/261,031